TOXICOLOGICAL EVALUATION OF REALISTIC EMISSIONS OF SOURCE AEROSOLS (TERESA): APPLICATION TO POWER PLANT-DERIVED $PM_{2.5}$

Semi-Annual Technical Progress Report

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ABSTRACT

This report documents progress made on the subject project during the period of September 1, 2007 through February 28, 2007. The TERESA Study is designed to investigate the role played by specific emissions sources and components in the induction of adverse health effects by examining the relative toxicity of coal combustion and mobile source (gasoline and/or diesel engine) emissions and their oxidative products. The study involves on-site sampling, dilution, and aging of coal combustion emissions at three coal-fired power plants, as well as mobile source emissions, followed by animal exposures incorporating a number of toxicological endpoints. The DOE-EPRI Cooperative Agreement (henceforth referred to as "the Agreement") for which this technical progress report has been prepared covers the performance and analysis of field experiments at the first TERESA plant, located in the Upper Midwest and henceforth referred to as Plant 0, and at two additional coal-fired power plants (Plants 1 and 2) utilizing different coal types and with different plant configurations.

During this reporting period, fieldwork was completed at Plant 2, located in the Midwest. The following scenarios were completed:

- July 19-22: POS (oxidized + SOA)
- July 25-28: PONS (oxidized + neutralized + SOA)
- August 8-13: P (primary)
- August 14-15: POS
- August 16-17: POS (MI rats)
- August 28-31: OS (oxidized + SOA, without primary particles)
- September 1-4: O (oxidized, no primary particles)
- September 6-9: S (SOA, no primary particles)
- September 19-22: PO (oxidized)

Results indicated some biological effects with some scenarios. Also during this reporting period, the annual meeting of the TERESA Technical Advisory Committee was held at the Harvard School of Public Health in Boston.

During the next reporting period, data analyses will continue for Plant 2 as well as for pooled data from all three plants. Manuscripts documenting the overall project findings will be prepared for submission to the peer literature. Preliminary planning will begin for the mobile source component of the research (funded through the Harvard-EPA Center for PM Health Effects), scheduled to take place in 2008.

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1.0 INTRODUCTION

The TERESA study investigates the role played by specific emissions sources and components in the induction of adverse health effects by examining the relative toxicity of coal combustion and mobile source (gasoline and/or diesel engine) emissions and their oxidative products. The work is a significant improvement over previous studies to investigate the toxicity of coal combustion-derived particulate matter by virtue of several highly innovative and unique design features. First, all toxicological studies of coal combustion emissions to date (some of which have shown biological effects) have used primary emissions, ie. coal fly ash (e.g. MacFarland *et al*., 1971; Alarie *et al.*, 1975; Raabe *et al*., 1982; Schreider *et al.*, 1985). The relevance of primary emissions to human population exposure is unclear, since primary PM emissions are now very low with the widespread introduction of particulate controls on power plants. It is the secondary particulate matter formed from SO_2 and NO_x in stack emissions as well as any residual primary PM that is of interest. No efforts to consider and account for secondary atmospheric chemistry have been made to date. By examining aged, atmospherically transformed aerosol derived from stack emissions, TERESA will enable the determination of the toxicity of emissions sources in a manner that more accurately reflects the exposure of concern. In addition, the atmospheric simulation component of the project will allow the investigation of the effect of different atmospheric conditions on the formation and toxicity of secondary PM. Second, the primary PM used in the studies to date has typically been generated through the use of pilot combustors in a laboratory setting. There is concern that pilot combustors may not accurately mimic stack emissions due to differences in surface to volume ratios and thus time-temperature histories. The fact that TERESA involves assessment of actual plant emissions in a field setting is an important strength of the study, since it directly addresses the question of representativeness of emissions.

The study involves on-site sampling and dilution of coal combustion emissions at three coal-fired power plants, as well as mobile source emissions. Emissions are introduced into a reaction chamber to simulate oxidative atmospheric chemistry, and both primary and secondary materials are extensively characterized, including NO_2, SO_2, ozone, NH_3, hydrocarbons, particle number and mass (including ultrafines), sulfate, nitrate, elemental/organic carbon (EC/OC), ammonium, and metals. Test atmospheres containing depleted emissions and emission oxidative products are utilized in two toxicological assessment steps, the first utilizing normal laboratory rats, and the second consisting of a comprehensive toxicological evaluation in a rat model of susceptible individuals. This last step includes telemetric methods for the assessment of cardiac function.

The primary objective of the project is to evaluate the potential for adverse health effects from ambient exposure to realistic coal-fired power plant emissions. Secondary objectives of the study are to: (1) evaluate the relative toxicity of coal combustion emissions and mobile source emissions, their secondary products, and ambient particles; (2) provide insight into the effects of atmospheric conditions on the formation and toxicity of secondary particles from coal combustion and mobile source emissions through the simulation of multiple atmospheric conditions; (3) provide information on the impact of coal type and pollution control technologies on emissions toxicity; and (4) provide insight into toxicological mechanisms of PM-induced effects, particularly as they relate to susceptible subpopulations. The study findings will help to answer questions regarding which constituents of PM are responsible for the negative health outcomes observed, the likely sources of these constituents, and the degree to which further regulation of PM will improve human health.

The DOE-EPRI Cooperative Agreement for which this technical progress report has been prepared involves the analysis and interpretation of the field data collected at the first power plant (henceforth referred to as Plant 0, located in the Upper Midwest), followed by the performance and analysis of similar field experiments at two additional coal-fired power plants (Plants 1 and 2) utilizing different coal types and with different plant configurations. The Agreement also includes a comparison of the toxicity of coal power plant emissions, mobile source emissions and concentrated ambient particles (CAPs). Animal exposure experiments to evaluate the toxicity of mobile source emissions and CAPs are also part of the overall TERESA program, but will be performed by the project team independently of the Agreement.

2.0 EXECUTIVE SUMMARY

Activities conducted during this reporting period (September 1, 2006 through February 28, 2007) focused on completing fieldwork at Plant 2 and continuing analysis and processing of all exposure characterization and toxicological data.

At Plant 2, located in the Midwest, the following scenarios were completed:

- July 19-22: POS (oxidized + SOA)
- July 25-28: PONS (oxidized + neutralized + SOA)
- August 8-13: P (primary)
- August 14-15: POS
- August 16-17: POS (MI rats)
- August 28-31: OS (oxidized + SOA, without primary particles)
- September 1-4: O (oxidized, no primary particles)
- September 6-9: S (SOA, no primary particles)
- September 19-22: PO (oxidized)

Exposure characterization data for Plant 2 have been processed and are reported in this progress report. In addition, most of the toxicological results from Plant 2 are reported.

Overall progress on the Project tasks is shown in the Table below. Note that the scheduled completion date for the Project has been extended due to a number of technical delays. We now anticipate completion of the project by December 31, 2008.

Technical Progress - 42 months

Task #	Description	Planned % completed	Actual % completed
1	Complete Study at Upper Midwest Power Plant	100%	100%
2	Field Study at Power Plant #1	100%	100%
3	Field Study at Power Plant #2	100%	100%
4	Relative Toxicity of Coal Plant Emissions, Mobile Sources, and CAPs	100%	0%
5	Preparation of Peer-Reviewed Journal Articles	100%	40%
6	Project management and reporting	100%	65%

Priorities for the next reporting period (March 1, 2007 – August 31, 2007) include:

- Completion of laboratory and toxicological analyses for Plant 2
- Completion of statistical analyses for all three plants
- Preparation of manuscripts for submission to the peer-reviewed literature

3.0 EXPERIMENTAL

A detailed description of the experimental setup and methods development is not provided in this report as these topics were covered extensively in prior semiannual reports. However, there was a change to the emissions sampling system at Plant 2, which is described below.

Emissions Sampling System

The previous emissions extraction system used for the Plant 1 did not work at Plant 2 due to high humidity conditions resulting from a wet Flue Gas Desulfurization (FGD) scrubber that caused water to condense inside the sampling probe and aspirator. Therefore, a new extraction system was designed to extract stack emissions by overcoming the water condensation problem. The dilution sampling scheme employed at Plant 2 was intended to dry out the stack emissions, and involved the introduction of hot, dry, and particle-free air into the sampling probe, mixing with the stack emissions, and extraction of the emissions using an aspirator. The dilution ratio was tuned by the controlling the amount of hot, dry air. The relationship between the dilution ratio and primary PM mass was investigated from September 23 – 25, 2006, when primary PM mass concentrations were relatively constant. A good correlation (R^2=0.98) indicates that the new extraction system was able to overcome the water condensation problem.

4.0 RESULTS AND DISCUSSION

4.1 Exposure Characterization

Animal exposures were carried out between July and September, 2006, as summarized below in Table 1. Note the following naming convention introduced to succinctly describe the scenarios:

- P = primary PM
- PO = primary PM + oxidized emissions
- POS = primary PM + oxidized emissions + SOA
- PONS = primary PM + oxidized, neutralized emissions + SOA
- OS = oxidized emissions + SOA (without primary particles)
- O = oxidized emissions (without primary particles)
- S = SOA (with ambient particle-free air, without primary particles)

The control scenarios (OS, O, and S) were completed to support the better interpretation of the exposure results.

Table 1. Summary of Plant 2 exposure scenarios and experiments.

Exposure Round	Code	Scenario	Dates	Animal Model
1	POS	Oxidized + SOA	July 19-22, 2006	Normal Rats
2	PONS	Oxidized + Neutralized + SOA	July 25-28, 2006	Normal Rats
3	P	Primary	August 8-13, 2006	Normal Rats
4	POS	Oxidized + SOA	August 14-15, 2006	Normal Rats
5	POS	Oxidized + SOA	August 16-17, 2006	MI Rats
6	OS	Oxidized + SOA (no primary particles)	August 28-31, 2006	Normal Rats
7	O	Oxidized (no primary particles)	September 1-4, 2006	Normal Rats
8	S	SOA (no primary emission)	September 6-9, 2006	Normal Rats
9	PO	Oxidized	September 19-22, 2006	Normal Rats

Continuous Measurements

Continuous data are provided in Table 3. Exposure parameters measured included RH, temperature, PM mass, ozone, NO, NO_2, SO_2, and particle count. For Plant 2, a TSI 8520 Dust Trak was used instead of the R&P TEOM used at Plant 1, due to serious damage during shipping. The continuous PM measurement was only used to verify that the exposure PM mass concentration was relatively constant during the exposures. Figure 5 provides the continuous PM concentration for a typical exposure day (12:30 through 18:30). The spikes are caused by the opening of animal chambers and integrated samples at the beginning and the end of the animal exposure test. Continuous PM mass by the Dust Trak is not reported here, due to a weak overall relation (R^2=0.44) between Dust Trak measurements and integrated PM mass concentrations. The plant had a very different pattern of particle number concentration, compared to the result for Plant 1. Particle number concentrations for the primary particle scenario (P) were much higher at Plant 2 (55,947 cm^{-3}) than at Plant 1 (910 cm^{-3}). The number concentration decreased somewhat with complexity of the scenarios, suggesting coagulation processes. The "S" control scenario (SOA without primary emissions), showed the lowest particle number of all the scenarios at Plant 2 (7,574 cm^{-3}). At the exposure end, the average SO_2 concentration for exposure round 1 included one exposure day with extremely high SO_2 concentrations attributed to an FGD scrubber problem that resulted in higher SO_2 concentrations for both ambient air and stack emissions. Temperature was steadily maintained at an average value of 23^{o}C and RH varied from 26.5% to 66.6%. Also, as specifically required for the toxicological tests, the gas concentrations for ozone, NO_x (NO and NO_2) and SO_2 were kept below 50 ppb, except for exposure round 1 (Table 3).

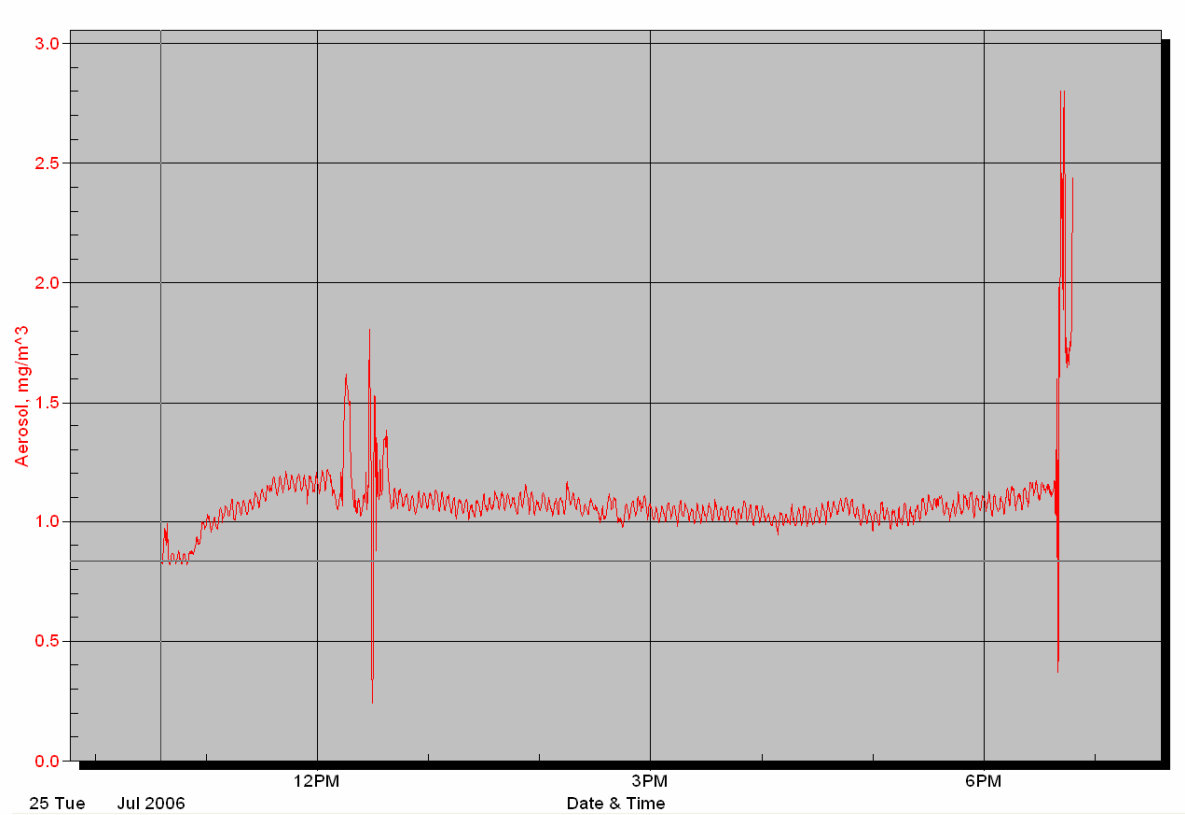


Figure 1. Exposure PM concentrations for a typical exposure day, Plant 2.

Integrated Measurements

To verify integrated mass concentration, two duplicate measurements were conducted simultaneously for primary PM entering the first reaction chamber. Results showed very good agreement (slope = 1.00, R^2 = 1.00) between the two measurements. Integrated measurements obtained are shown in Table 4. Some suspect data were found and eliminated from data analysis for exposure round 4. The suspect data included two integrated PM mass samples, one ion species data of two data for the round, which is likely due to leaking and/or tearing problem during the sampling. PM mass concentrations ranged from 62 µg/m^3 for the SOA scenario without primary emission (S) to 279 µg/m3 for one of the oxidized emissions + SOA scenarios (POS). Three exposure rounds conducted for the oxidized emissions + SOA scenario (POS) showed different mass concentrations (279 and 250 µg/m^3 including one missing data) at the same condition except for primary emission (Table 4). It is important to note that there is a fair amount of day-to-day variation in mass concentration, even within a given exposure round, because different ratios for SO_2 vs. NO occurred for different days, resulting in different amounts of sulfate produced in the first reaction chamber. In addition, because the primary PM mass is considerably higher than at the Plant 1 and because it also varies somewhat from day to day, it is another factor causing variation in measured PM mass. The primary PM also seems to originate

mainly from the wet FGD scrubber rather than the coal combustion boiler. Therefore, this difference is likely due to the inherent variation in the power plant operation.

Total sulfate concentrations showed a similar pattern with PM mass, ranging from 1 $\mu g/m^3$ for the SOA scenario without primary emissions (S) to 89 $\mu g/m^3$ for the oxidized emissions + SOA scenarios (POS) at exposure round 1. In terms of acid and neutral sulfate, most of sulfate for un-neutralized scenarios (PO and POS) consists of acid sulfate, while most of sulfate for neutralized scenario (PONS) consists of neutral sulfate with higher nitrate and ammonium concentration. Significant PM mass and acid sulfate concentrations were found in the primary particles (P). It might be originated from the scrubber using limestone slurry at the plant. Nitrate was low in all scenarios, except the neutralized scenario (PONS). Ammonium was also similarly low in all scenarios except the neutralized run (PONS).

In terms of EC and OC concentrations, we can construct a group from all the scenarios with SOA (POS, PONS, OS, and S scenarios) and another group without SOA (P, PO, and O scenarios). The "S" scenarios showed much higher OC concentrations (56 to 101 $\mu g/m^3$), representing secondary organic aerosol from the second reaction chamber, than the non-"S" scenarios (9 to 16 $\mu g/m^3$). However, there was some overestimation of OC concentration against PM mass concentration. It is also important to note that these positive artifacts may have been introduced into the system due to volatile organic compounds (VOCs) in the clean (particle free) room air used for flushing the series of 2 denuders (refer to experimental section of previous reports). In addition, EC was not detected in any of the scenarios (Table 4).

Elemental data obtained from integrated measurements performed at Plant 2 are presented in Table 5. The complete dataset is presented instead of summary statistics to clearly depict substantial day-to-day variations recorded for the elemental concentrations which again provide insight about the inherent variations attributed to plant operation. The values are bold for those that are at least twice the uncertainty values. However, there may be some usefulness for values less than twice the uncertainty, so they are also included in the table. Also, note that each sample has a different set of uncertainty values because with XRF, the uncertainty for each element is related to corrections for interference by a different set of elements, and the distribution of element magnitudes is different for each sample. All elements had low concentrations except for sulfur and the most prominent of these were: Al, Si, Cl, Ca, Fe, and Se (marker element for coal combustion). However, we were questioned the accuracy of all XRF measurements when the S concentrations by XRF method were compared with sulfate concentrations by ion chromatography. Even though the S was the most abundant and significant element of all elements, the XRF accuracy is most likely not reliable, since the correlation was quite poor (R^2=0.19).

Exposure PM Composition

Exposure PM composition obtained for each exposure day is shown in Figure 6. Exposure days with a missing data were excluded from the calculation. Unidentified components can be explained by organic materials, un-analyzed ion species, and uncertainty. As shown in the figure, exposure days within each scenario showed similar patterns. Major components were sulfate (25 to 50%) and OC (5 to 50%) for all scenarios with a large variation. Ammonium, nitrate, and non-S elements accounted for a few percent of total PM mass, as minor components. Each component was calculated for percentage to total PM mass, and then averaged across each scenario. For the primary particle scenario (P), the PM was composed of 17% acid sulfate and

29% neutral sulfate, which seem to originate from the wet scrubber. Also, OC accounted for 22% of the total PM mass concentration. The composition of the oxidized emissions (PO) and oxidized emissions + SOA (POS) scenarios were similar except OC, showing that the PM was composed of 35% and 30% acid sulfate, 5% and 6% neutral sulfate, and 5% and 29% OC. PM in the PONS scenario was composed of 1% acid sulfate and 34% neutral sulfate, showing the neutralization of this material by ammonia, and 28% OC. For the control scenarios, acid and neutral sulfate accounted for 15% and 8% of total PM mass for the OS scenario, and 37% and 10% of total PM mass for the O scenario, respectively. For the SOA scenario without primary emissions (S), OC overestimated total PM mass, possibly due to a VOC artifact.

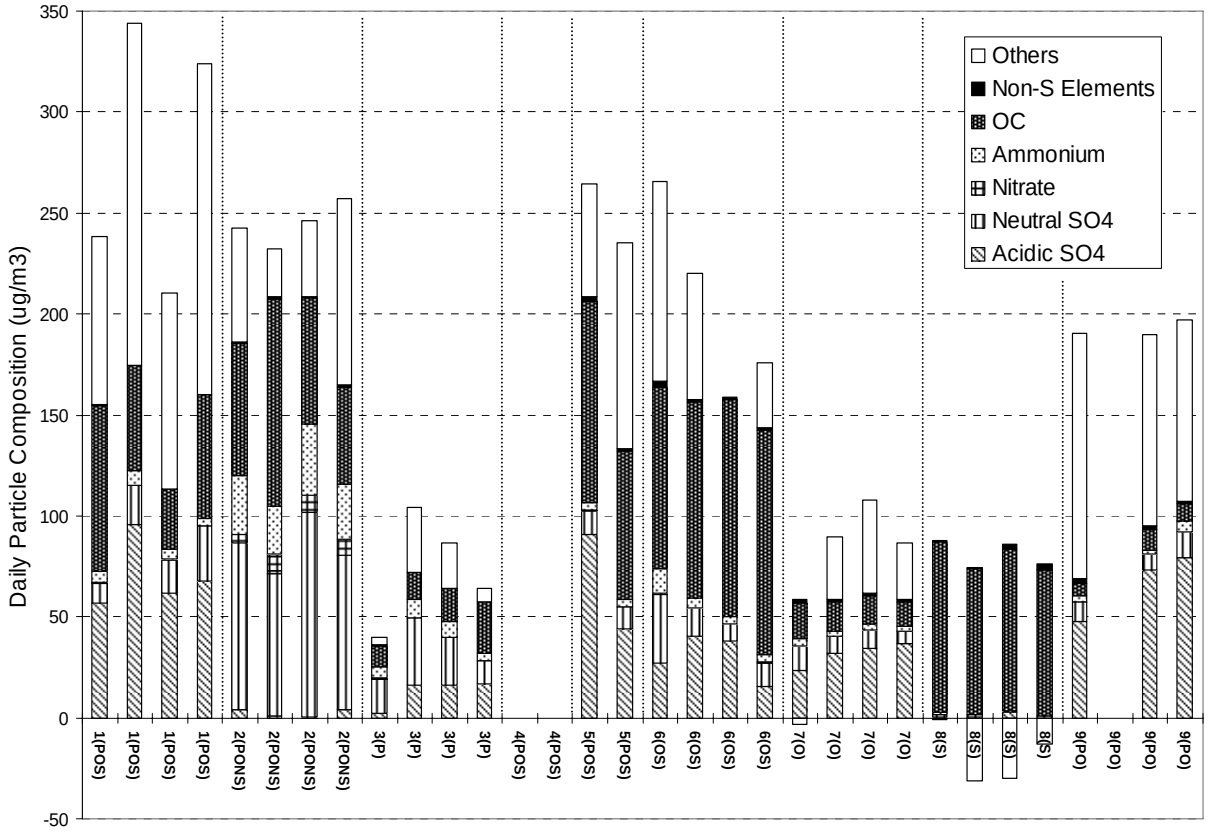


Figure 2. PM composition for each exposure day, Plant 2.

Table 2. Continuous measurements during experimental runs at Plant2, July-September, 2006.

Exposure Parameter	Round 1 (POS)	Round 2 (PONS)	Round 3 (P)	Round 4 (POS)	Round 5 (POS)	Round 6 (OS)	Round 7 (O)	Round 8 (S)	Round 9 (PO)
Temperature (˚C)	23.0±0.9	21.9±0.3	23.3±1.0	24.6±1.2	24.5±0.5	24.5±1.5	23.5±0.0	23.9±0.3	23.4±1.2
RH (%)	66.6±12.0	53.0±17.1	56.1±17.6	48.6±17.7	31.4±15.6	47.5±10.2	38.7±10.3	36.0±2.0	26.5±11.6
O_3(ppb)	24.1±11.9	15.2±6.6	8.9±3.3	31.5±5.4	37.0±3.8	19.9±3.3	18.3±4.2	21.0±2.4	28.8±8.8
NO(ppb)	6.7±0.8	6.3±0.3	7.5±2.4	7.3±1.7	8.1±2.7	7.5±3.8	5.8±0.1	8.4±0.7	9.4±2.2
NO_2(ppb)	5.5±0.3	3.7±2.2	4.4±0.8	3.5±1.2	4.5±1.8	2.0±0.4	2.5±0.7	3.8±1.0	3.7±0.7
SO_2(ppb)	111.9±109.5[1)]	23.1±6.9	38.9±15.7	25.7±1.4	41.6±4.2	15.1±6.6	33.9±16.4	27.3±9.8	34.7±7.1
PM Count (# cm^{-3})	38,400±5,386	38,483±3,651	55,947±11,77	42,867±1,656	42,116±13,65	35,959±6,290	29,294±2,392	7,574±1,598	69,372±8,523

Note. [1)]The mean value contains a higher SO_2 episode; Rounds 1-3 and 6-9 were four days in duration; Rounds 4 and 5 were two days in duration; Values expressed as Mean±SD.

Table 3. Integrated measurements during experimental runs at Plant2, July-September, 2006.

Exposure Parameter	Round 1 (POS)	Round 2 (PONS)	Round 3 (P)	Round 4 (POS)	Round 5 (POS)	Round 6 (OS)	Round 7 (O)	Round 8 (S)	Round 9 (PO)
Mass(μg m^{-3})	279.0 ± 64.7	244.4 ± 10.2	73.8 ± 28.0	NA[2)]	249.7 ± 20.5	205.1 ± 47.9	84.9 ± 22.0	62.2 ± 18.4	193.1 ± 3.5
Primary Mass[1)](μg m^{-3})	71.5 ± 12.4	41.1 ± 11.2	73.8 ± 28.0	50.2 ± 12.5	56.2 ± 1.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	13.6 ± 5.8
Total Sulfate(μg m^{-3})	88.7 ± 21.0	85.1 ± 12.8	34.0 ± 13.3	71.7 ± NA[3)]	78.6 ± 33.5	47.3 ± 14.6	40.6 ± 3.8	1.3 ± 0.4	77.0 ± 17.6
Neutral Sulfate(μg m^{-3})	18.3 ± 7.1	82.6 ± 13.4	21.2 ± 9.2	6.1 ± NA[3)]	11.2 ± 0.7	17.0 ± 11.6	8.9 ± 2.3	0.8 ± 0.6	10.4 ± 2.8
Acid Sulfate(μg m^{-3})	70.4 ± 17.3	2.5 ± 2.0	12.8 ± 7.1	65.6 ± NA[3)]	67.4 ± 32.8	30.3 ± 11.6	31.7 ± 5.8	1.0 ± 1.3	66.6 ± 16.8
Nitrate(μg m^{-3})	0.3 ± 0.3	7.5 ± 2.3	0.2 ± 0.3	0.0 ± NA[3)]	0.3 ± 0.4	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
Ammonium(μg m^{-3})	5.4 ± 1.6	28.9 ± 4.8	6.7 ± 2.6	2.5 ± NA[3)]	3.7 ± 0.1	6.3 ± 4.2	2.6 ± 0.7	0.4 ± 0.2	3.2 ± 1.7
OC(μg m^{-3})	56.1 ± 21.7	69.6 ± 23.0	16.2 ± 6.4	72.3 ± 51.8	86.4 ± 18.8	101.2 ± 9.6	14.5 ± 2.2	77.3 ± 6.1	9.2 ± 2.6
EC(μg m^{-3})	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SO_2(ppb)	69.4 ± 68.8	12.6 ± 4.2	24.4 ± 6.2	7.2 ± 6.2	25.1 ± 2.7	11.3 ± 3.9	25.0 ± 14.5	22.7 ± 8.0	23.2 ± 5.1
HNO_3(ppb)	0.3 ± 0.4	1.1 ± 1.0	0.1 ± 0.2	0.3 ± 0.4	0.3 ± 0.2	0.4 ± 0.5	0.0 ± 0.1	0.2 ± 0.2	0.1 ± 0.2
HONO(ppb)	3.4 ± 0.9	3.3 ± 3.0	3.1 ± 0.5	1.7 ± 0.0	1.5 ± 2.2	0.9 ± 1.7	0.1 ± 0.2	0.7 ± 0.9	0.2 ± 0.4
NH_3(ppb)	3.1 ± 3.7	4.9 ± 4.6	0.4 ± 0.4	3.6 ± 5.1	0.0 ± 0.0	8.8 ± 10.4	0.3 ± 0.6	3.4 ± 2.8	2.3 ± 4.6

Note. [1)]Primary PM mass entering the first reaction chamber; [2)]Not available two data; [3)]Not available one data; Rounds 1-3 and 6-9 were four days in duration; Rounds 4 and 5 were two days in duration; Values expressed as Mean±SD.

Table 4. Elemental concentrations (μg/m^3) for each exposure day at Plant 2, July-September, 2006.

Round	Na	Mg	Al	Si	S	Cl	K	Ca	Ti	V	Cr	Mn
1(POS)	0.000	0.000	0.102	**0.130**	**19.360**	0.000	**0.017**	**0.132**	0.000	0.000	0.000	0.000
1(POS)	0.000	0.000	**0.198**	0.038	**18.781**	0.000	0.000	0.000	0.001	0.000	0.000	0.000
1(POS)	0.000	0.000	**0.166**	**0.136**	**14.860**	0.000	0.000	**0.214**	0.003	0.000	0.000	0.000
1(POS)	0.000	0.000	0.131	**0.195**	**12.870**	0.000	**0.017**	**0.086**	**0.013**	0.001	**0.009**	0.000
2(PONS)	0.000	0.000	0.080	0.028	**16.279**	**0.185**	0.003	**0.086**	0.001	0.000	0.000	0.000
2(PONS)	0.374	0.000	**0.444**	**0.523**	**31.573**	**0.132**	0.000	**0.087**	0.001	0.000	0.002	0.007
2(PONS)	0.000	0.000	**0.429**	**0.258**	**31.951**	**0.124**	0.000	**0.056**	**0.015**	0.001	0.002	0.000
2(PONS)	0.000	0.000	**0.475**	**0.507**	**36.434**	**0.214**	0.000	0.010	0.000	0.000	0.000	0.001
3(P)	0.000	0.000	0.061	**0.095**	**10.130**	0.000	0.000	0.011	0.000	0.001	0.001	0.000
3(P)	0.000	0.004	**0.225**	**0.091**	**25.479**	0.000	0.000	**0.045**	0.000	0.002	0.000	0.000
3(P)	0.000	0.000	0.000	0.002	**9.733**	0.000	0.000	0.005	0.001	0.002	**0.008**	0.000
3(P)	0.000	0.000	0.052	0.000	**11.748**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4(POS)	0.000	0.000	**0.214**	**0.280**	**22.579**	0.000	0.000	**0.272**	0.007	0.003	0.000	0.000
4(POS)	0.805	0.008	0.030	**0.279**	**6.605**	**0.030**	**0.028**	**0.215**	0.000	**0.006**	0.000	0.000
5(POS)	1.445	0.000	0.039	**0.647**	**62.955**	**0.119**	**0.113**	**0.536**	0.016	**0.025**	**0.013**	0.000
5(POS)	0.824	0.152	0.111	**0.193**	**13.985**	**0.049**	**0.027**	**0.397**	0.007	0.004	0.001	0.000
6(OS)	0.880	0.630	0.123	**0.684**	**27.701**	**0.160**	**0.033**	**0.255**	**0.019**	**0.005**	0.002	0.000
6(OS)	0.650	0.000	0.000	**0.527**	**23.164**	0.010	**0.004**	**0.029**	0.000	**0.008**	0.000	0.000
6(OS)	**0.857**	0.197	0.035	**0.499**	**13.555**	**0.050**	**0.002**	**0.040**	0.000	**0.004**	0.000	0.000
6(OS)	0.959	**0.357**	0.008	**0.395**	**10.991**	**0.155**	0.000	**0.017**	0.002	0.002	0.001	0.000
7(O)	**1.183**	0.120	0.074	**0.361**	**10.230**	**0.017**	**0.029**	**0.263**	**0.012**	0.003	0.003	0.000
7(O)	0.400	**0.265**	**0.213**	**0.529**	**9.978**	0.001	**0.034**	**0.073**	**0.013**	0.003	0.000	0.000
7(O)	0.978	0.046	**0.195**	**0.289**	**12.171**	0.000	**0.055**	**0.100**	0.008	**0.010**	0.001	0.000
7(O)	0.878	**0.270**	0.093	**0.133**	**13.560**	0.000	**0.002**	**0.035**	0.002	**0.005**	0.002	0.000
8(S)	0.399	**0.226**	0.004	**0.161**	**0.875**	**0.098**	**0.000**	**0.034**	0.000	0.001	0.000	0.001
8(S)	0.000	0.000	**0.078**	**0.148**	**0.750**	**0.073**	**0.010**	**0.112**	0.000	0.003	0.002	0.000
8(S)	**2.136**	0.279	0.029	**0.193**	**0.834**	**0.099**	**0.013**	**0.069**	**0.015**	0.005	0.005	0.000
8(S)	**2.223**	**0.255**	0.036	**0.087**	**0.815**	**0.089**	**0.002**	**0.024**	0.003	**0.006**	**0.005**	0.000
9(PO)	0.864	0.187	0.000	**1.460**	**11.670**	0.000	**0.031**	**0.087**	0.000	**0.006**	0.000	0.001
9(PO)	0.076	0.000	**0.130**	**0.933**	**8.842**	0.005	**0.000**	**0.036**	0.004	**0.007**	0.000	0.000
9(PO)	**0.994**	**0.370**	0.027	**0.579**	**8.239**	0.000	**0.015**	**0.072**	0.000	**0.006**	0.000	0.000
9(PO)	0.669	0.109	0.000	**0.854**	**15.978**	0.000	**0.006**	**0.027**	0.000	0.004	0.002	0.000
LOD	0.147	0.008	0.037	0.000	0.018	0.058	0.045	0.004	0.003	0.003	0.003	0.001

Table 4. (contd.) Elemental concentrations (μg/m^3) for each exposure day at Plant 2, July-September, 2006.

Round	Fe	Ni	Cu	Zn	Se	Br	Sr	Ag	Cd	Ba	La	Hg
1(POS)	**0.041**	0.000	0.001	**0.012**	0.008	**0.017**	0.000	0.000	0.000	0.006	0.001	0.000
1(POS)	0.001	0.000	0.000	0.001	**0.021**	0.007	0.003	0.007	0.000	0.005	0.000	0.010
1(POS)	0.001	**0.005**	0.002	**0.010**	**0.012**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1(POS)	**0.033**	0.003	0.000	**0.011**	**0.009**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2(PONS)	**0.030**	0.002	0.001	**0.041**	**0.027**	0.008	0.000	0.000	0.000	0.000	0.000	0.000
2(PONS)	**0.162**	0.000	**0.011**	**0.026**	**0.034**	0.011	0.000	0.088	0.013	0.008	0.023	0.000
2(PONS)	**0.018**	0.000	0.001	0.001	**0.047**	0.000	0.000	0.000	0.015	0.000	0.013	0.000
2(PONS)	**0.060**	0.000	0.000	**0.015**	**0.066**	**0.014**	0.000	0.000	0.000	0.000	0.009	0.000
3(P)	0.006	0.000	0.000	**0.013**	**0.023**	0.000	0.000	0.005	0.004	0.005	0.007	0.000
3(P)	0.000	0.000	0.003	**0.004**	**0.037**	0.000	0.000	0.000	0.000	0.000	0.000	0.001
3(P)	0.004	0.004	**0.005**	**0.021**	**0.023**	0.000	0.001	0.032	0.000	0.009	0.000	0.000
3(P)	0.007	0.001	0.000	**0.011**	0.008	0.000	0.008	0.005	0.000	0.000	0.000	0.000
4(POS)	**0.102**	0.000	0.000	**0.007**	**0.010**	0.003	0.000	0.027	0.000	0.005	0.000	0.000
4(POS)	**0.044**	**0.016**	0.000	**0.014**	0.001	0.004	0.001	0.000	0.006	**0.007**	0.003	0.000
5(POS)	**0.231**	0.016	0.000	**0.071**	**0.061**	0.019	0.000	0.000	0.000	**0.028**	0.000	0.000
5(POS)	**0.040**	0.005	0.000	0.013	0.004	0.004	0.000	0.000	0.018	0.000	0.000	0.000
6(OS)	**0.140**	0.004	0.000	**0.024**	0.000	**0.012**	0.000	0.002	0.035	0.000	0.000	0.000
6(OS)	**0.026**	0.000	0.000	**0.022**	0.006	0.005	0.006	0.006	0.020	**0.008**	0.000	0.000
6(OS)	**0.009**	0.005	0.000	0.005	0.001	**0.009**	0.000	0.003	0.013	**0.007**	0.001	0.006
6(OS)	0.009	0.012	0.000	**0.014**	0.001	0.005	0.000	0.000	0.000	0.000	0.000	0.000
7(O)	**0.075**	0.012	0.000	**0.039**	**0.013**	0.005	0.000	0.000	0.000	0.000	0.000	0.008
7(O)	**0.073**	0.007	**0.016**	**0.038**	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.016
7(O)	**0.059**	0.011	0.000	**0.015**	0.000	0.000	0.000	0.000	0.009	**0.018**	0.000	0.000
7(O)	**0.012**	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.015**	0.000	0.000
8(S)	0.017	0.005	0.000	0.010	0.001	0.002	0.000	0.005	0.020	0.000	0.000	0.000
8(S)	0.016	0.006	0.000	**0.019**	0.000	0.000	0.000	0.008	0.025	0.003	0.000	0.000
8(S)	0.004	0.015	0.000	0.002	0.001	0.000	0.000	0.008	0.000	0.006	0.000	0.000
8(S)	0.008	0.001	0.000	**0.012**	0.000	0.000	0.000	0.000	0.024	0.000	0.006	0.000
9(PO)	0.008	**0.016**	0.000	**0.033**	**0.014**	0.004	0.000	0.000	0.007	**0.030**	0.000	0.000
9(PO)	**0.061**	0.011	0.000	**0.018**	0.006	0.000	0.000	0.000	0.010	0.000	0.006	0.000
9(PO)	**0.052**	0.011	0.000	**0.013**	0.006	0.004	0.000	0.000	0.024	0.000	0.003	0.003
9(PO)	**0.013**	0.008	0.000	**0.015**	0.003	0.000	0.000	0.000	0.000	**0.009**	0.000	0.000
LOD	0.000	0.002	0.007	0.008	0.000	0.000	0.000	0.000	0.000	0.032	0.025	0.001

4.2 Toxicological Assessment

Use of Animals

Animal experiments were conducted in relationship to each of the scenarios described earlier in this report. On each day, 10 male Sprague-Dawley rats were exposed for 6 hours; 5 were exposed to the experimental exposure aerosol for that day, and 5 underwent sham exposures to filtered air. On each day, all animals in both groups had their continuous breathing pattern monitored with the BUXCO system. Of the 5 animals in each group, 2 animals from each group had cardiac and pulmonary *in vivo* chemiluminescence analyzed immediately after exposure, and tissue was collected from each site for subsequent analyses of thiobarbituric acid reactive substances (TBARS). The remaining animals of each group had inflammatory responses of the lungs assessed at 24 hours post-exposure by bronchoalveolar lavage (BAL) including cell analyses as well as fluid studies for protein and released enzymes. Animals on alternate days not used for BAL had lung and heart tissues collect for histopathological assessment. Animals assessed for either BAL or histology also had blood samples taken for complete blood count and differential (CBC&DIFF). Thus, for each scenario, there were repetitions over 4 days resulting in a total of 20 rats in the experimental exposure group and 20 rats in the sham exposure group. For specific outcomes, therefore, the number of animals per group were 20 and 20 for BUXCO, 8 and 8 for chemiluminescence/TBARS, 6 and 6 for histopathology, 6 and 6 for BAL, and 12 and 12 for CBC&DIFF. At Plant 2, the total number of animals studied was 148 in the experimental exposure group, and 147 in the sham exposure group.

Chemiluminescence and TBARS

For all scenarios, there were slight differences in chemiluminescence and TBARS, but none of these in individual comparisons by ANOVA reached significant differences. Figures 3-7 below illustrate these results.

Figure 3.

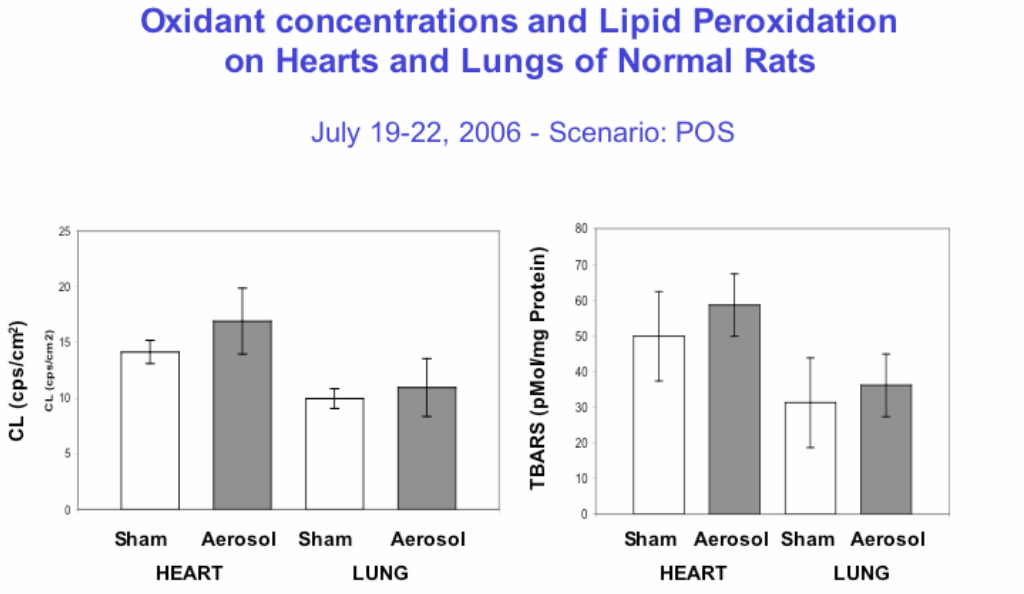


Figure 4.

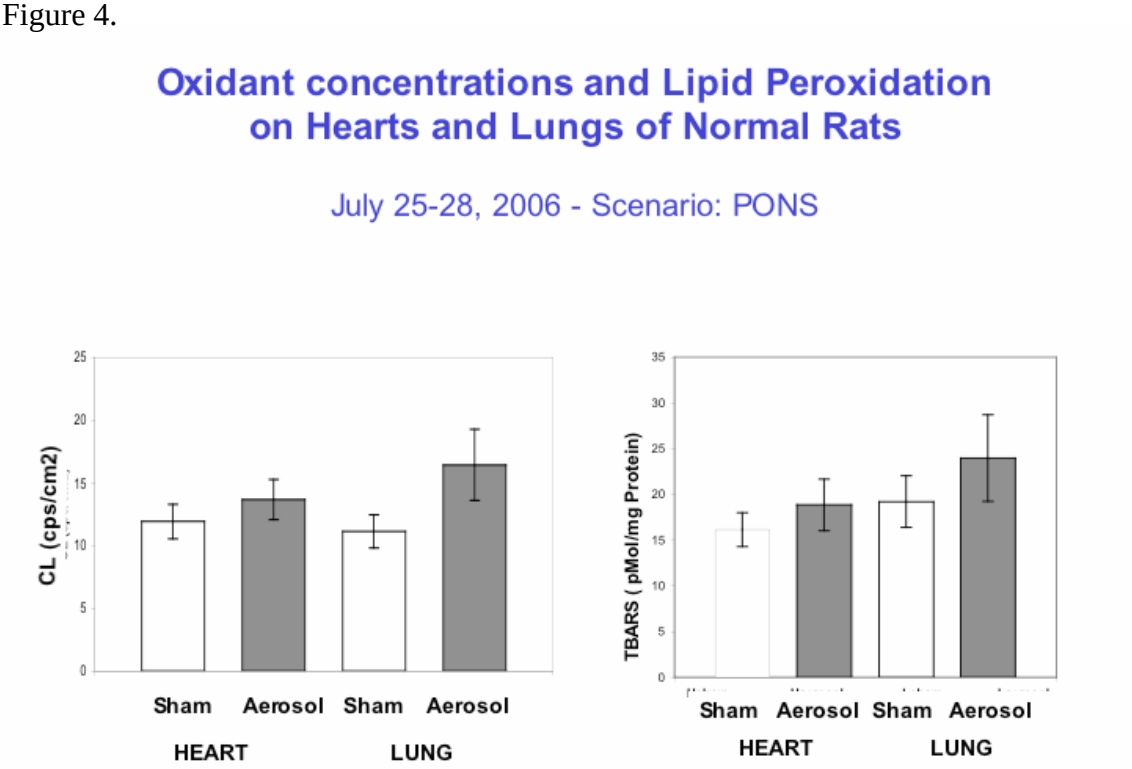


Figure 5.

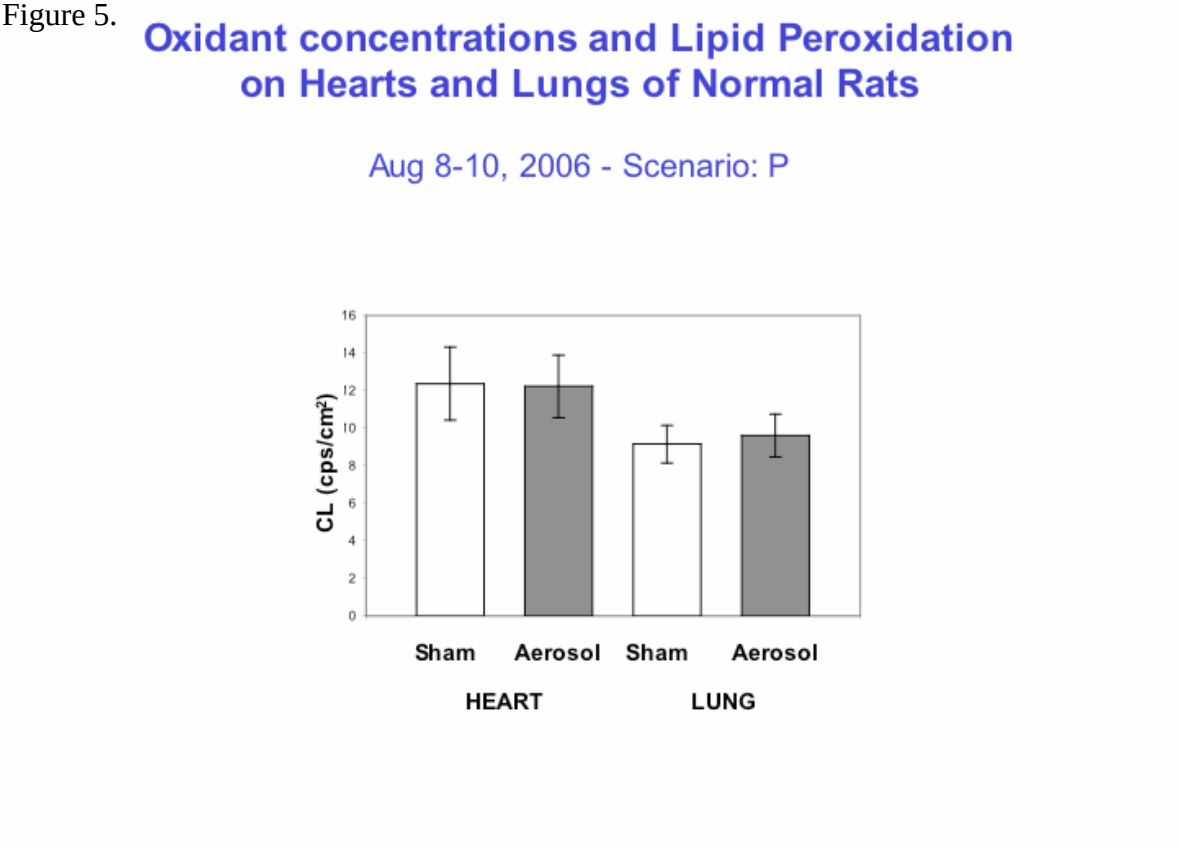


Figure 6.

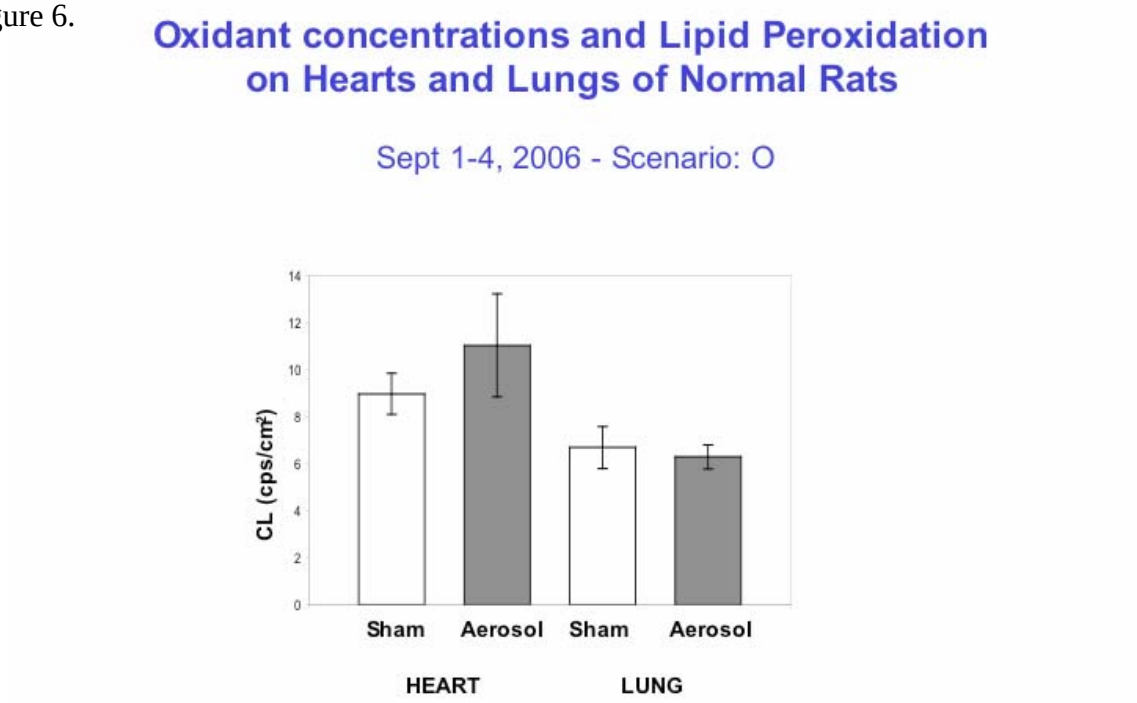
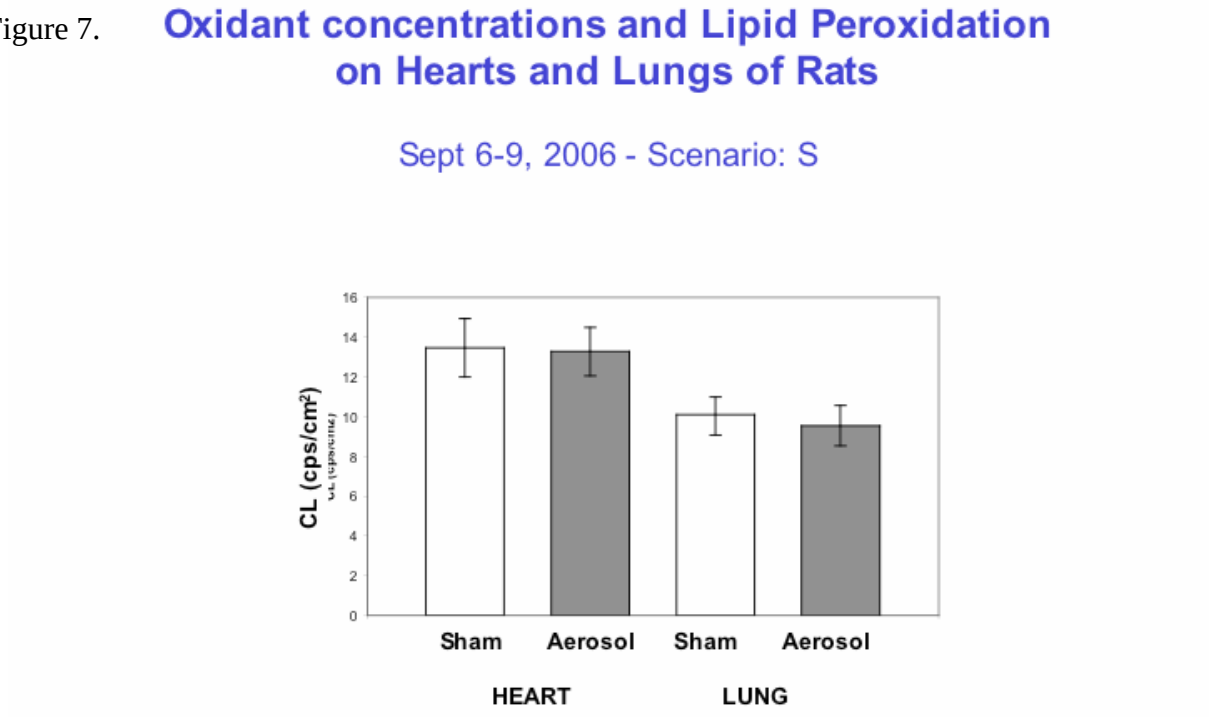


Figure 7.

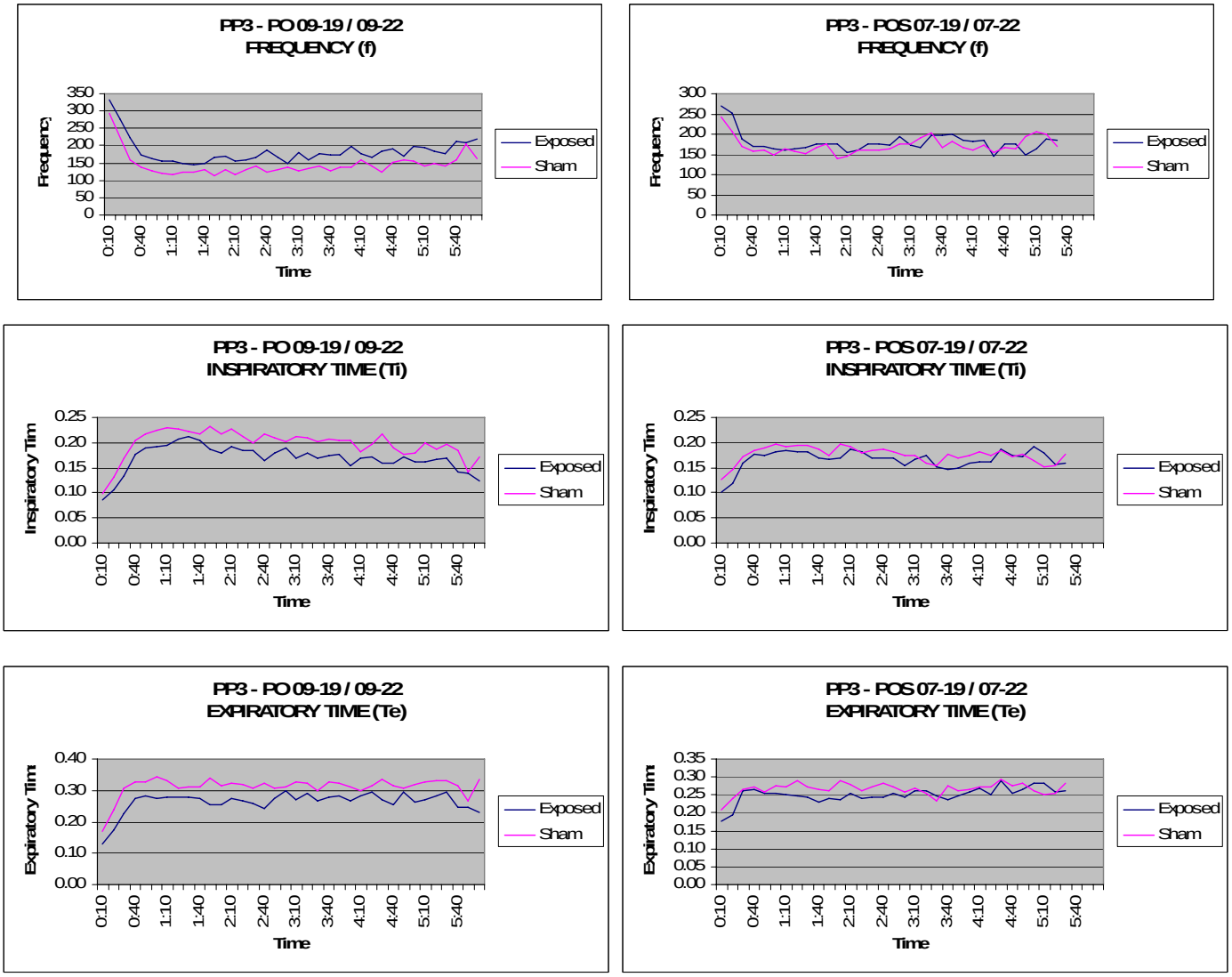


There are additional TBARS analyses to be completed, as well as a meta-analysis of all three power plants in relationship to these outcomes.

Respiratory Parameters

Extensive analyses of respiratory parameters have been completed. The data in the figures below illustrate the change in a given respiratory parameter throughout the 6 hour exposure and represent the mean of all animals for a scenario and particular response. Thus, each line shown is the mean of 20 determinations within a scenario.

Figure 8. Respiratory results for Plant 2.



Statistical assessments of the data are shown in Table 5. Those patterns lacking overlap in the graphical display were found to be significant where overlap of the experimental and sham exposures were not statistically significant. At Plant 2, the PO scenario was found to increase respiratory rate with corresponding decreases in the time of inspiration and expiration. These changes suggest a slight irritation that changes the breathing pattern to a rapid shallow pattern.

Table 5. Plant 2 respiratory data summary showing p-values and direction of the change.

Scenario Parameter	PO	POS
RR	↑ p<0.002	↑ ns
TV	↑ ns	↑ ns
Ti	↓ p=0.003	↓ ns
Te	↓ p<0.002	↓ns
Penh	↓ p=0.01	↓p=0.03

We have also begun preliminary regression analyses to better understand the exposure atmosphere components that may be influencing the observed respiratory outcomes. Table 6 shows the data using this regression approach on the data from Plant 2.

Table 6. Linear regression of respiratory rate, TV, Ti, and Te in relation to selected pollutants, showing p-value and direction of the change, Plant 2.

Pollutant	**Rate**	**TV**	**Ti**	**Te**
H2SO4	**NS**	**NS**	**↓0.02**	**NS**
OC	**↑0.005**	**NS**	**↓0.002**	**↓0.002**
Fe	**↑0.02**	**NS**	**↓0.004**	**↓0.002**

From these data we can see that the respiratory irritation that changed breathing pattern at Plant 2 was related largely to the concentrations of organic carbon and iron.

Complete Blood Counts and BAL

No evidence of a change in any CBC parameter was found in the blood at any plant comparing exposure with sham animals. However, meta-analyses of pooled data from all three plants revealed a few results which are not clearly explained. Monocytes (absolute number) in the CBC decreased with increasing Si concentration (P=<0.00001) in the analysis of data of all plants**.** Lymphocytes (absolute number) in the CBC decreased in all plants in association with increasing OC concentration (p=0.006), and increased in all plants in association with increasing

Si concentration (p=0.003). The meaning of these results in these meta-analyses are not clear at this time.

No BAL parameter showed a significant difference in any of the plants comparing exposure with sham. Total Cell Count increased in the all plant analyses and was positively and significantly associated with every measured exposure parameter. No specific cell type accounted for this increase.

Acute Myocardial Infarction Studies

The objective of these studies was to assess the toxicity of the experimental aerosol on the propensity to arrhythmias during the early period following myocardial infarction. Thus, an acute myocardial infarction was produced by coagulating the anterior descending coronary artery of the rat during thoracic surgery. The chest was closed, the rat recovered, and within 12 hours of the infarction, was exposed to the experimental or sham aerosol. Continuous electrocardiograms (ECGs) were recorded in unrestrained animals with implanted telemetry units. The ECG signal was exported to Matlab for analysis. Beats were automatically labeled by the software and verified by the investigator. Premature ventricular beats (PVBs) were identified. Analysis of arrhythmia data used Poisson regression in a GEE framework to estimate the effect of exposure during each hour accounting for within-subject correlation.

Results from Plants 1 and 2 are compared. Table 7 illustrates the number of animals available for analyses at each plant. At Plant 2, although two attempts were made to do studies using the myocardial infarction model, only one set was successful. The second time the experiment was run near the end of our stay at the plant, problems with the unit of the plant from which we were withdrawing emissions precluded doing the exposure within the window of vulnerability that has been defined with this model. Thus, we have limited data from Plant 2. Numbers of animals analyzed are shown in Table 7.

Table 7. Numbers of animals with usable data in the myocardial infarction model.

	Plant 1	**Plant 2**	**Total**
Sham	**14**	**2**	**16**
Exposed	**15**	**5**	**20**

At Plant 1, overall, the average rate of PVBs across entire exposure period was 93.5% greater in exposed rats than in sham rats (p=0.041). An hourly analysis showed no significant differences in the first hour (p=0.84). In sham rats, the rate of PVBs decreased over time, whereas the rate of PVBs was greater at each time point in exposed rats. This difference was statistically significant after 4 hr of exposure (p=0.049) and marginally significant after 5 hr (p=0.079).

At Plant 2, overall, the average rate of PVBs across the entire exposure period was 60.0% *lower* in exposed rats than sham rats (p=0.035). There were not enough data to compare effects by hour. In pooled analyses, the data from Plant 2 negated the findings of Plant 1. Given the small amount of data from Plant 2, each plant will be considered separately. However, in the

Plant 2 data, there appeared to be an increase in atrial arrhythmias. We will reanalyze Plant 1 data to determine if this observation is important at this location.

Studies to be Completed

In the next period, we will complete respiratory analyses, carry out additional TBARS analyses, and complete the histological and BAL analyses. Supraventricular arrhythmia studies will be completed including going back over Plant 1 animals. Additional statistical analyses of the data will be done to understand compositional differences and the outcomes found. We anticipate completing a series of publications to be submitted to the same journal, which will include papers covering:

- Exposure analyses
- Chemiluminescence and TBARS analyses
- Respiratory data
- BAL, CBC, and histology
- Myocardial infarction model studies
- Summary paper

Writing these papers has begun and preliminary exploration of such a series in one journal has been discussed with one editor. We anticipate that several editors will be contacted before a final decision is made on where this series of papers for a special issue of a journal will be submitted.

Summary of Toxicological Findings

The toxicological results at the second plant had some similarities, but also substantial differences, from the other plants. Overall, there were fewer positive results at Plant 2 than at Plant 1. Combining all three power plants has the potential to provide significant insight into the toxicity of the aged and atmospherically chemically reacted effluents of power plants. There are statistically and biologically significant responses observed at Plant 1 and 2. We need to complete our analyses to determine the implications of these findings.

5.0 CONCLUSIONS

Significant progress was made on the Project during this reporting period. We completed fieldwork at Plant 2, and most of the laboratory and data analyses.

Priorities for the next reporting period (March 1, 2007 – August 31, 2007) include:

- Completion of laboratory and toxicological data analyses for Plant 2, located in the Midwest.
- Completion of statistical analyses for all three plants
- Preparation of manuscripts for submission to the peer-reviewed literature